Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Linkage Inversion Assembled Nano-Aptasensors (LIANAs) for Turn-On Fluorescence Detection

Table of Contents:

I. Synthetic Procedures

a) Materials and Methods

Gold (III) chloride hydrate ($HAuCl_4 \cdot 3H_2O$, ≤ 99.9%), Silver nitrate ($AgNO_3$, ≤ 99.9%), L-ascorbic acid (≥99.5%), Sodium borohydride ($NaBH_4$, 99%), 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC, ≥99%), sulfo-N-hydroxysulfosuccinimide (sulfo-NHS, ≥98.5%) and Hexadecyltrimethylammonium bromide (CTAB, ~99%) were purchased from Sigma-Aldrich. Ochratoxin A (OTA) and warfarin standards were purchased from Sigma-Aldrich. Ochratoxin B (OTB) was purchased from Santa Cruz Biotechnology Canada. Carboxyl QDs 525 (Green CdSe/ZnS) and Carboxyl QD 655 (Red CdSeTe) were purchased from Life Technologies, Burlington, ON Canada. DNA synthesis reagents and modifiers were purchased from Glen Research. Standard support columns and acetonitrile were purchased from BioAutomation. Ultra High Purity 5.0 argon was purchased from Praxair. All buffers

were prepared with Millipore Milli-Q deionized water at 18MΩ. All molecular biology grade electrophoresis chemicals were purchased from BioShop Canada (Burlington, Ontario). TEM images were recorded using FEI Tecnai F20 FETEM in Carleton University, Chemistry Dept. UV/Vis absorption spectra were obtained using a CARY 300 Bio spectrophotometer (Varian, USA).

Photoluminescence was measured with a HORIBA Fluorolog 3 instrument. A high-speed Sorvall legend micro 21R (Thermo electron corporation) centrifuge was used for the centrifugation of the solution. Modified aptamers were prepared in-house using a MerMade 6 DNA Synthesizer. 5′-5′ reverse phosphoramidite linker DNA was purchased from University Core DNA Services, University of Calgary, Calgary, Alberta.

b) Buffers

LIANA 1	Buffer A	10 mM HEPES, 120 mM NaCl, 5 mM KCl, and 5 mM $MgCl_2$	pH 7.0
LIANA 2	Buffer B	10 mM Na_2HPO_4, 2 mM KH_2PO_4, 2.7 mM KCl, and 137 mM NaCl	pH 7.4

c) Gold nanoparticle (AuNP) synthesis

Procedures for colloidal gold nanoparticle preparation were essentially the same as those developed by Grabar *et al*,[1] except the decrease in the volume of $HAuCl_4$ to sodium citrate, since higher $HAuCl_4$ concentration leads to larger colloids.[2] All glassware used for AuNP synthesis was cleaned by soaking in aqua regia (3:1 mixture of concentrated HCl/HNO_3) for 15 minutes followed by thorough rinsing with deionized water. A 250 mL Erlenmeyer flask was used to mix 98 mL of deionized water and 2 mL of 50 mM $HAuCl_4$ to a final concentration of 1 mM $HAuCl_4$. The solution was heated to boiling with stirring. Once boiling, 10 mL of 38.8 mM sodium citrate was added. Following the change in suspension colour from pale yellow to dark blue and finally to a wine red, heating was continued for an additional 20 minutes. The flask was removed from the heat and allowed to cool to room temperature with continued stirring. The nanoparticles were quantified by UV-Vis.

d) Functionalization of AuNPs with thiol-modified aptamer

Surface modification of gold nanoparticles with thiol-modified aptamer was carried out by addition of thiol-modified aptamer to colloidal gold in water with stirring in the dark for 2 days. 1.2 µL of 0.1 µM thiol-modified aptamer was added to 2.5 µL of 460 pM colloidal gold solution. To remove the unbound thiol-modified aptamer, the samples were centrifuged. The precipitate obtained was further purified by dispersion in buffer solution and subsequent centrifugation with a speed of 14 000 rpm for 30 minutes. This process was repeated twice to remove any unbound thiol-modified aptamer. Aptamer functionalized AuNPs were stored in buffer at 4°C.

e) Synthesis of gold nanorods (AuNRs)

The AuNRs used in this study were synthesized by a surfactant-stabilized, seedless one-step technique following a procedure reported in the literature.[3] $HAuCl_4$ (20 mM), $AgNO_3$ (0.1 mM), and ascorbic acid (0.1 M) were mixed in 10 mL of 0.1 M aqueous cetyl trimethylammonium bromide (CTAB) solution. 40 mL of 1.6 mM $NaBH_4$ was used as the reducing agent to generate AuNRs. Finally, the nanorods were purified by centrifugation at 14 000 rpm for 30 minutes to remove the excess metal ions and CTAB molecules in the supernatant solution for terminating the self-assembly growth (most of the excess surfactant must be separated from the AuNRs, otherwise coupled plasmon peaks cannot be observed upon the addition of linker DNA). The AuNRs (aspect ratio = 3.5) had plasmon absorption peaks around 520 and 650 nm.

f) Functionalization of AuNRs with thiol-modified aptamer

The terminal ends of AuNRs appear to be more chemically reactive than the sides of nanorods.[4] Caswell *et al* reported biotin-disulfides that can preferentially anchor onto the ends of the gold nanorod surface. The thiol groups bind to the Au (111) crystal facets on the nanorod ends, while CTAB bilayers protect the nanorod sides from thiol binding.[5] 3 µL of 230 pM AuNRs were mixed with 1.2 µL of 0.1 µM thiol-modified aptamer and stirred gently for two hours. In this step, thiol groups of the modified aptamer are selectively attached onto the terminal edges of AuNRs. AuNRs were then washed and finally, the resulting conjugates were collected by centrifugation at 14 000 rpm for 30 minutes then resuspended in buffer.

g) Functionalization of carboxyl QDs with amine-modified aptamers

The amine modified aptamer (1.12.2 and A08) was conjugated to the carboxyl CdSeTe 655 QDs and CdSe/ZnS 525 core/shell QDs using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysulfo-succinimide (S-NHS) as cross-linking reagents.[6] QDs (920 pM, 5 µL) were mixed with 0.5 µL of EDC (25eq to carboxyl QDs) and 1.45 µL of S-NHS (50eq to carboxyl QDs) in phosphate buffered saline (PBS, pH 7.4) total volume 450 µL. After shaking for 30 min, amine-modified aptamer (0.1 µM, 1.2 µL) was added and placed on a shaker at room temperature for an additional two hours. In this way, the amide linkage can form through the amino modification of the aptamer and the active carboxyl of QDs. In order to remove the excess small molecules, e.g., EDC and S-NHS, the resulting samples were centrifuged at 14 000 rpm for 20 minutes. A similar procedure was followed for the mixed aptamers (*1.12.2* and *A08min*) conjugated to the mixed QDs (CdSe/ZnS QDs and (CdSeTe).

h) Preparation of LIANA1 and LIANA2 samples

The LIANA1 samples were prepared by mixing the aptamer-functionalized AuNPs (460pM, 2.5µL, aqueous solution), the aptamer-functionalized QDs (920 pM, 5µL, aqueous solution) and *5'-5' linker* DNA (0.1 µM, 1.2µl) in buffer A. This solution was shaken and incubated for 1 hr at room temperature.

The LIANA2 samples were prepared similarly, except that aptamer-functionalized AuNRs (230pM, 3µL, aqueous solution), aptamer-functionalized QDs (460 pM green CdSe/ZnS 2.5µL; 460 pM red CdSeTe 2.5µL, aqueous) and *5'-5' linker* DNA (0.1 µM, 1.2µl) were mixed in buffer B. Similarly to LIANA1, the solution was shaken and incubated for 1 hour at room temperature. The same procedure was used for the preparation LIANA2 with different linker DNAs (*10-nt linker, 16-nt linker, 22-nt linker*).

i) Preparation of OTA-spiked complex extract

The extract was prepared by mixing 2 g each of sample (wheat, barley, corn, oats and malted barley) from Trilogy Analytical laboratory (reference samples) with 50 ml of deionized water, and shaking for 10 minutes. The mixture was centrifuged for 10 minutes and the supernatant was filtered through Whatman 1 filter paper and a syringe filter (PES 0.45 µm, 30 mm diameter). The clear solution was spiked with OTA to concentrations from $1x10^{-9}$ to $1x10^{-4}$ M (each in 1 mL).

j) Characterization of LIANA samples

HR TEM samples were prepared by drop casting 10 µL of the various LIANA samples onto a carbon coated copper grid. For CLSM measurements, 10 µL of the various LIANA samples were spotted onto glass slides. After drying overnight, samples were mounted in Fluoromount G from EMS (Hatfield,

PA) and covered with a high-performance coverslip (0.17 mm thick) from Zeiss (Toronto, Ontario, Canada). Images were acquired on Confocal Zeiss LSM510 META with a Plan-Apochromat 63×/1.4 Oil DIC objective and electronic zoom 5. Fluorescence was observed using the Argon/2 laser 488 nm excitation line with emission BP (Band Pass) 490−600 nm for CdSe/ZnS QDs and exciting laser DPSS 561 with emission LP (Long Pass) 650 nm for CdSeTe QDs.

k) Paper test preparation

Three rows of sample zones (~8 mm diameter circles) were prepared on unmodified Whatman 41 filter paper. The top row was left empty as a control lane of OTA samples. LIANA2 sample 1 µL in buffer B were spotted onto the bottom two rows of sample zones and left to dry for two minutes. 1 µL of 10^{-4} – 10^{-9} M OTA solutions in complex extract were spotted on to the top and bottom rows of sample zones. The middle row of sample zones were spotted with 1 µL of the matrix alone (matrix control). The paper test was then illuminated with a hand-held UV light (254 nm) and visualized with a Nikon camera (model: D7000).

l) Melting temperature (T_m)

Variable-temperature UV-Vis spectroscopy melting studies (T_m determination) were performed with a Varian Cary 300 Bio UV-Vis Spectrophotometry equipped with a 6x6 Peltier-Thermostatted Multicell Holder. For each sequence, 3 ml of aptamer was prepared at 2 µM in buffer. *A08min* was measured alone and in the presence of 2 µM of each linker DNA. Absorbance was monitored at 260 nm over three temperature ramps (Ramp 1 80°C - 20°C; Ramp 2 20°C - 80°C; Ramp 3 80°C - 20°C). The ramp rate was set at 0.5°C/min (slow) with a five minute hold between each temperature change. Melting temperatures were determined by fitting the change in absorbance at 260 nm relative to temperature using Standard Curves Analysis on SigmaPlot.

II. Figures

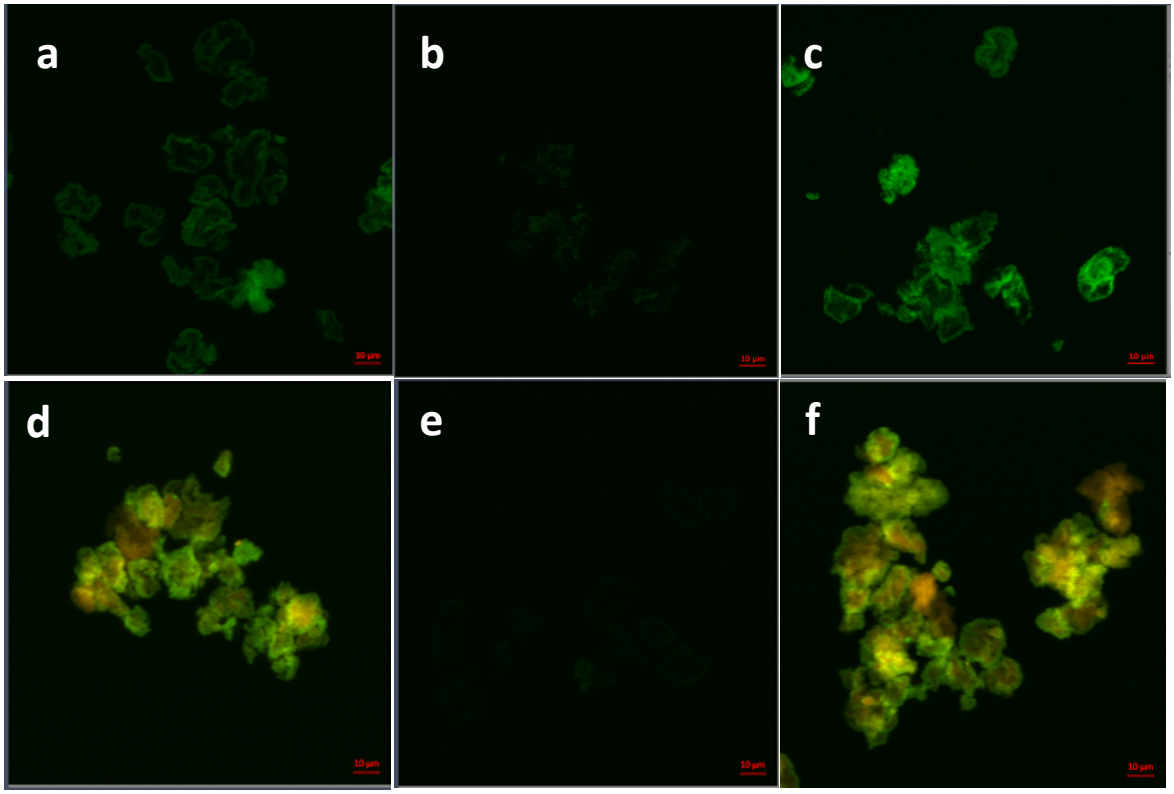

Figure S1. a) CLSM image of the mixture of aptamer-AuNPs and aptamer-QDs without the addition of linker DNA, showing green emission. b) Assembled structure of LIANA1 leads to AuNP quenching of QDs using the 5′-5′ linker DNAs (0.1 µM). c) After the addition of 1.8 µM of OTA, dispersion of LIANA1 and the return of the green emission is noted. d) A mixture of aptamer-AuNRs and aptamer-QDs (CdSeTe and CdSe/ZnS) displays red and green emission from the two QDs. e) Quenching of the fluorescence is observed after the addition of 5′-5′ linker DNA (0.1 µM) to form LIANA2. f) Disassembly of LIANA2 with OTA (1.8 µM) leads to the detection of both QD emission signals. Scale bar is 10 µm. LIANA1 and LIANA2 used the 1.12.2 and A08 OTA aptamers, respectively, for these images.

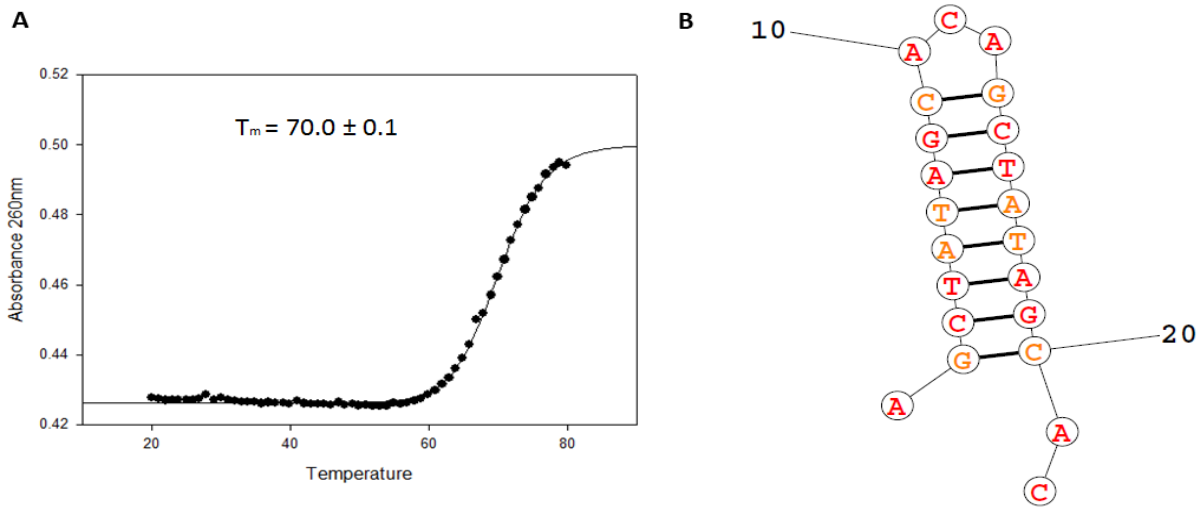

Figure S2: a) T_m data for *22-nt linker* from variable temperature UV-vis at 260 nm. **b)** Predicted secondary structure of *22-nt* from RNAstructure software.[7]

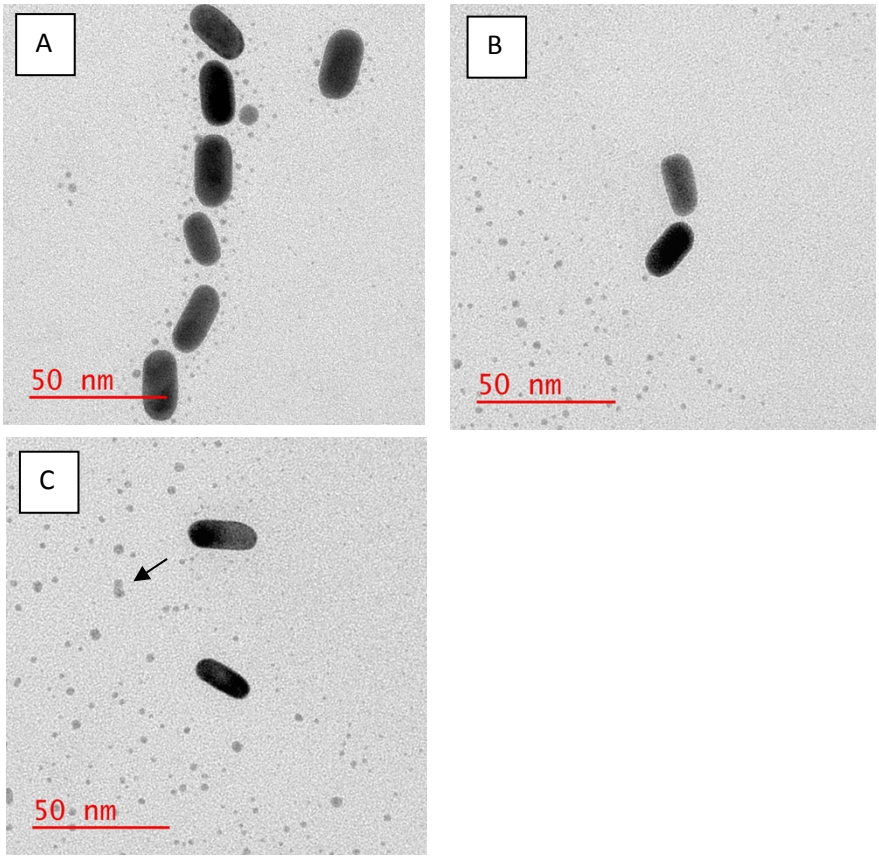

Figure S3: Assemblies observed during LIANA2 preparation. A) Example of a multi AuNR-QD assembly. B) Example of an AuNR-AuNR assembly C) Example of a QD-QD assembly (arrow).

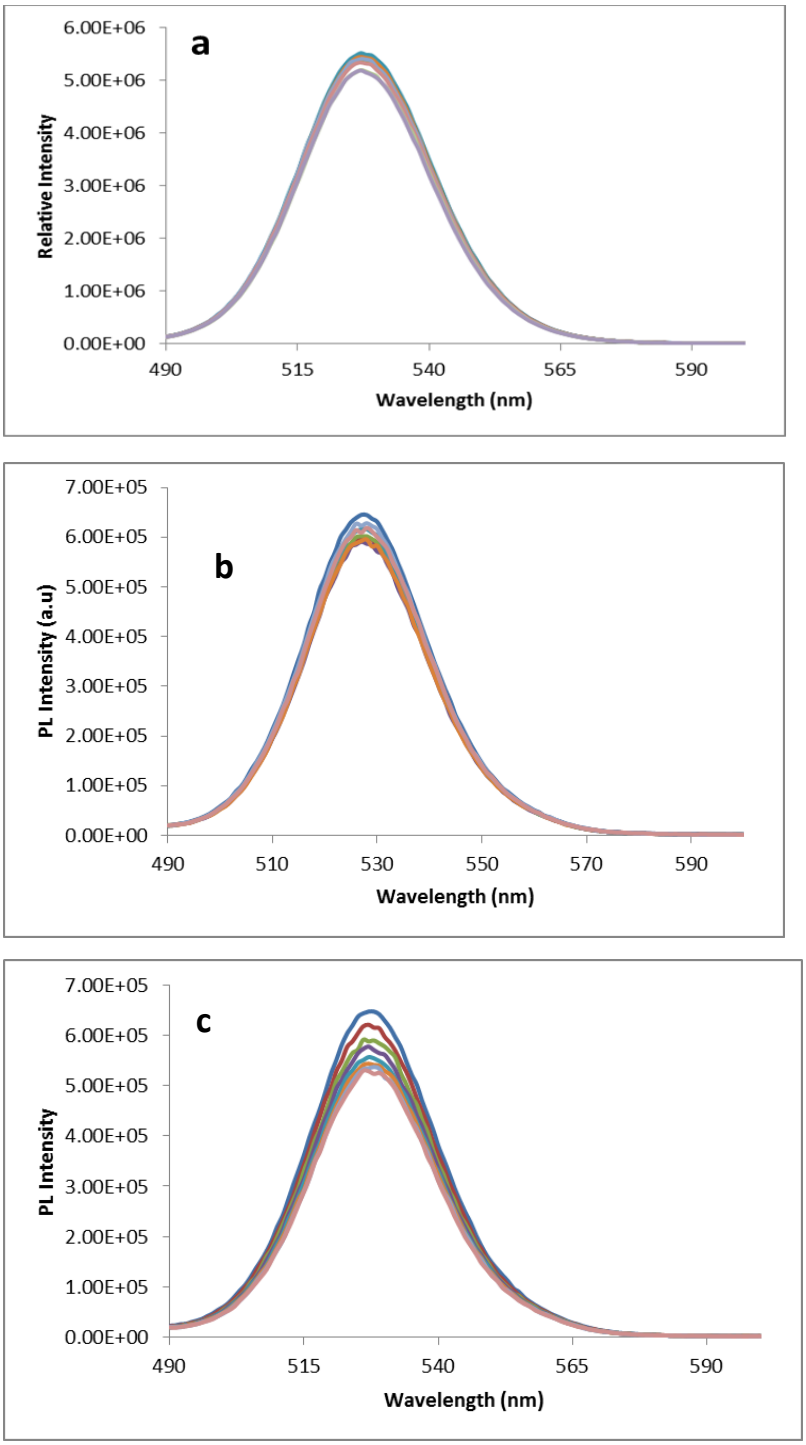

Figure S4. a) PL spectra of aptamer-QDs alone upon the addition of 0–1.8 µM of OTA. The intensity fluctuates slightly but there is no significant change. b) PL spectra of LIANA1 in aqueous solution upon the addition of 0–1.8 µM of OTB. There is a slight reduction in intensity. c) PL spectra of LIANA1 upon the addition of 0–1.8 µM of warfarin. There is a slight decrease in intensity with increasing concentration.

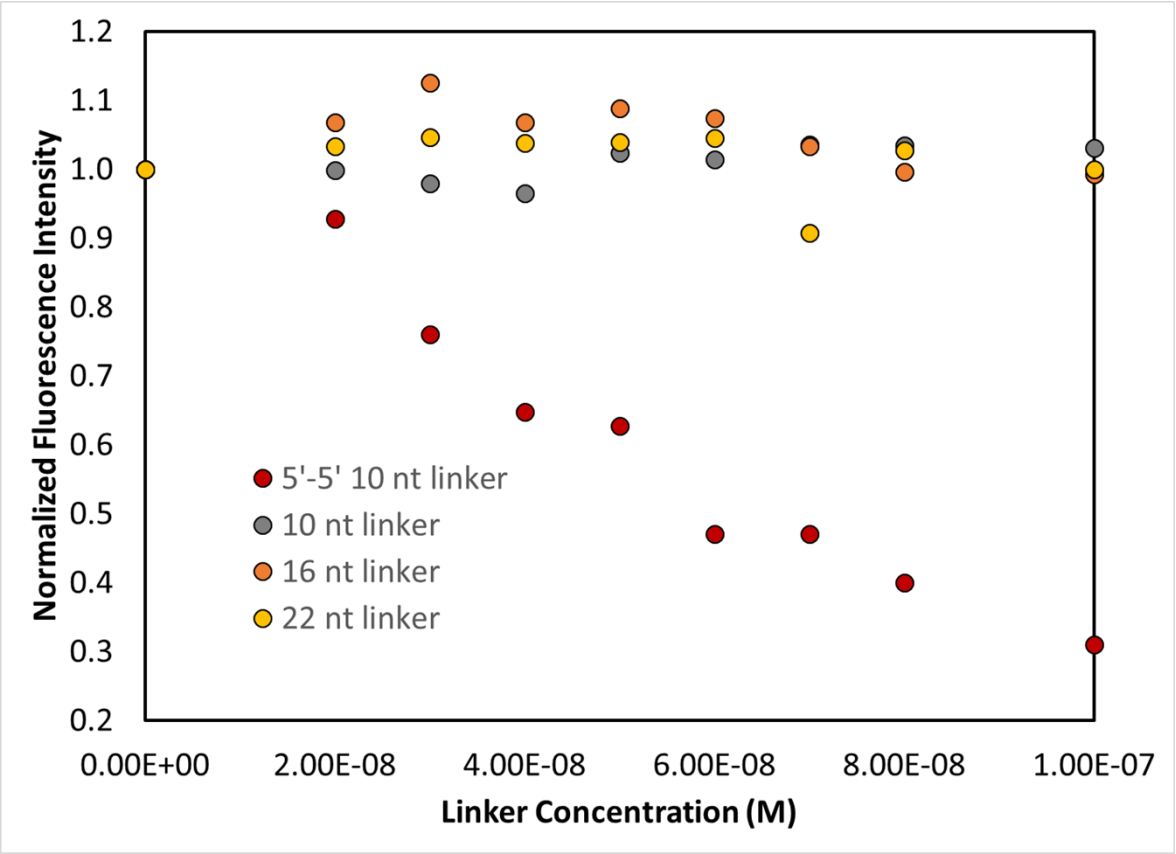

Figure S5: Comparison of normalized PL intensity at 530 nm of the aptamer-modified AuNRs and Aptamer-modified QDs in the presence of the various linkers. Increasing the 5'-5' linker concentration leads to quenching by the formation of the LIANA. The normal linkers of varying lengths show little evidence of quenching or assembly formation.

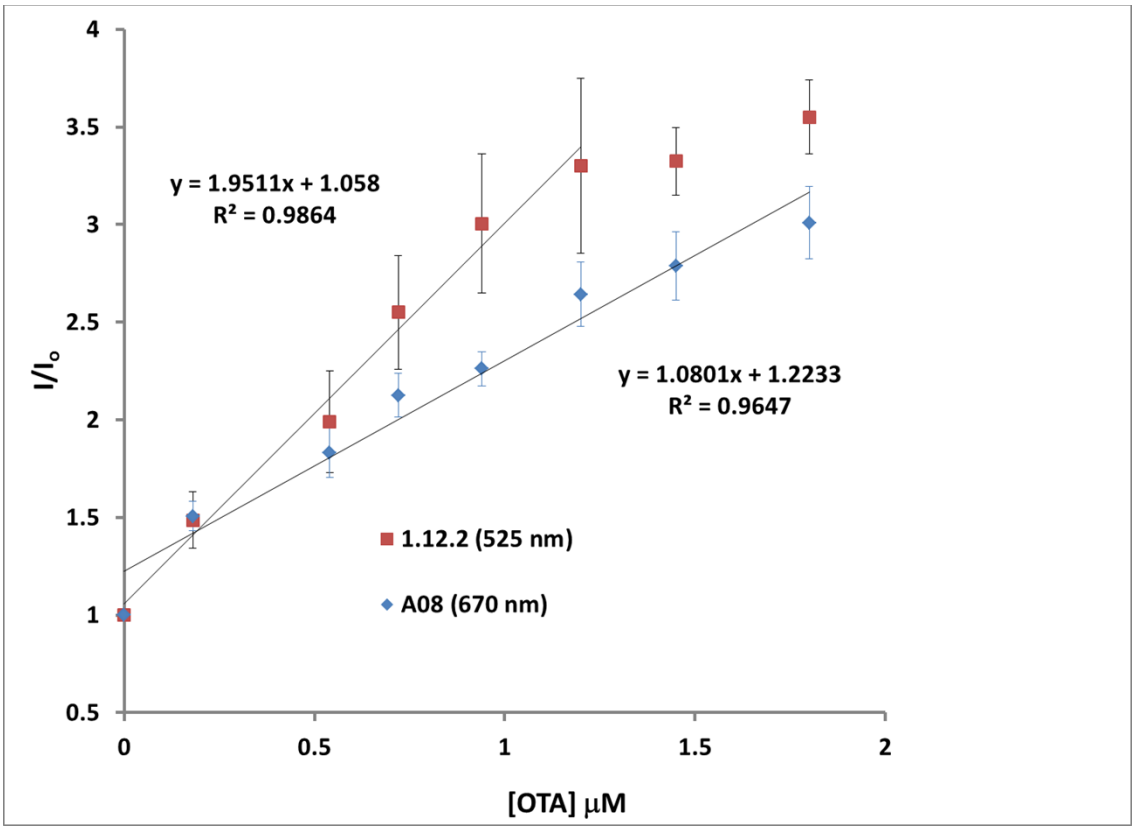

Figure S6: The LIANA approach is general and can be applied to other aptamer sequences. *1.12.2*-labelled green QDs and *A08*$_{min}$-labeled red QDs assembled into a LIANA2 complex show simultaneous fluorescence sensing of OTA with slightly different sensitivities.

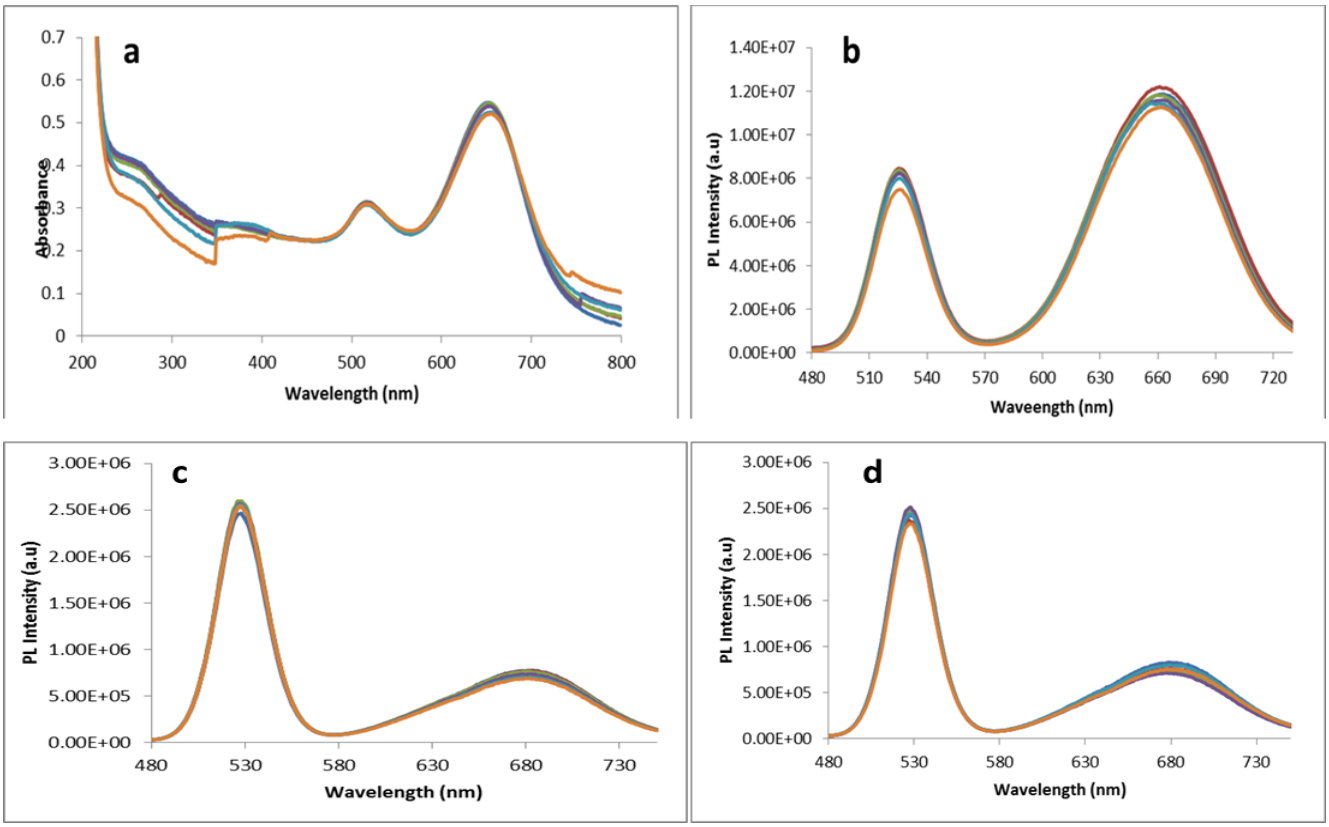

Figure S7. a) Longitudinal surface plasmon (LSP) absorption of aptamer-AuNRs alone upon the addition of 0–1.8 µM of OTA. The absorbance fluctuates slightly but there is no significant change. b) PL spectra of aptamer-QDs (green-QDs and red QDs) alone upon the addition of 0–1.8 µM of OTA. The intensity fluctuates slightly but not significantly. c) PL spectra of LIANA2 upon the addition of 0–1.8 µM of OTB. d) PL spectra of LIANA2 upon the addition of 0–1.8 µM of Warfarin. The intensity fluctuates slightly but there is no significant trend.

III. References

[1] K.C. Grabar, R.G. Freeman, M.B. Hommer, M.J. Natan, *Anal. Chem,* **1995**, *67*, 735-43

[2] G. Frens, *Nat. Phys. Sci.* **1973**, *241*, 20-22

[3] a) D.A. Walker, V.K. Gupta, *Nanotechnology*, **2008**, *19*, 435603 (9pp); b) N.R. Jana, *Small* **2005**, *1*, 875-82

[4] a) M. Gluodenis, C.A. Foss, *J. Phys. Chem. B,* **2002**, *106*, 9484-89. b) Y.W. Yuan, W. Jian, S.You, Z.Cheng, *Chem. Commun*, **2010**, *46*, 1332-34

[5] K.K. Caswell, J.N. Wilson, U.H.F. Bunz, C.J. Murphy, *J. Am. Chem. Soc.* **2003**, *125*, 13914-15

[6] a) J. Park, Y. Park, S. Kim, *ACS Nano*, **2013**, 7, 9416-9427 b) H. Jin, J. Nam, J. Park, S. Jung, K. Im, J. Hur, J.J. Park, J.M. Kim, S. Kim, *Chem. Commun.,* **2011**, *47*, 1758-1760.

[7] J.S. Reuter, D.H. Mathews, *BMC Bioinformatics,* **2010**, *41*, 471-474.